

**Research Article**

# The Investigation of Adrenomedulline Levels in Dogs with Heart Failure

Esra BULUT<sup>1</sup>, Özkan DURU<sup>2</sup>, Buğrahan Bekir YAĞCI<sup>1</sup>

<sup>1</sup>Kırıkkale University, Veterinary Faculty,  
Internal Medicine Department, Kırıkkale/  
Türkiye

<sup>2</sup> Kırıkkale University, Veterinary Faculty,  
Biochemistry Department, Kırıkkale/Türkiye

ORCID:0000-0001-9054-4973

ORCID: 0000-0002-8792-442X

ORCID: 0000-0002-7473-3579

## Correspondence

Kırıkkale University, Veterinary Faculty,  
Internal Medicine Department, Kırıkkale/  
Türkiye

E- mail: ebulutt94@gmail.com

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## Abstract

In this study, it was intention to investigate adrenomedulline (ADM) level in dogs with heart failure. Animal materials within the scope of the study; 20 diagnosis of heart failure have been referral to Kırıkkale Veterinary Faculty Education and Research hospital without age, gender and racial discrimination. At the same time, blood samples were taken from 10 healthy dogs with no heart failure (control group). Serum samples were obtained from blood samples; for the examination of adrenomedulline enzyme - connected immunosorbent test kit. In the assessments; while the ADM level was looked high in dogs with heart failure, it was not registered as high in the control group. As a result of the examinations, a significant increase in the value of adrenomedulline in dogs with heart failure.

**Keywords:** Adrenomedulline, heart failure, dog

## Introduction

Adrenomedullin (ADM) is a novel hypotensive and bioactive peptide originally discovered by Kitamaru et al in 1993 from pheochromocytoma tissue extract by monitoring cAMP elevation in rat platelets (Etöz and Büyükcöskün, 2005, Kato et al., 1996, Sakata et al., 1994). They named this peptide as adrenomedullin (ADM) because it is found not only in pheochromocytoma tissue but also in normal adrenal medulla (Etöz and Büyükcöskün, 2005). ADM, a compound originally isolated from extracts of human pheochromocytoma tissue, has also been identified as a neurohumoral factor with potent vasodilator activity (Kanno et

al., 2012). The main characteristic effect of ADM is to dilate blood vessels and reduce systemic arterial pressure, both endothelium-dependent and endothelium-independent (Etöz and Büyükcöskün, 2005, Beetowski and Jamroz, 2004). Reseraches report that ADM production is mainly produced from the lung, kidney, placenta, adrenal medulla and cardiovascular system (Kitamura et al., 2002, Voors et al., 2019, Geven, Kox and Pickkers, 2018). ADM, a peptide determined to have vasodilator and natriuretic effects, plays a role in the regulation of the cardiovascular system (Kanno et al., 2012). Adrenomedulline is a key hormone involved in the regulation of the endothelial barrier and vascular tone (Nagayama et al., 2000).

Disruption of the ADM system results in vascular leakage and systemic and pulmonary edema (Voors, et al., 2019). ADM is released from the vascular wall and acts as an autocrine or paracrine hormone to regulate vascular tone and blood pressure (Wong, et al., 2012). In vitro experiments have shown that ADM exerts multiple actions on cultured vascular cells, mostly protective or inhibitory against vascular damage and progression of arteriosclerosis (Kato, et al., 2005). In this study, it was aimed to determine the extent to which ADM, thought to have a direct relationship with heart failure, is affected in dogs with heart failure. Additionally, the goal was to ascertain whether ADM can be utilized as a diagnostic biomarker in the context of heart failure. Furthermore, it was considered that this investigation could shed light on the pathogenesis of the disease.

## Material and Method

Within the scope of the research, the animal material; 20 patient dogs of different ages, genders and breeds were brought to Kırıkkale University Veterinary Faculty Training and Research Hospital and diagnosed with heart failure (HF). Animals brought to Kırıkkale University Faculty of Veterinary Medicine Animal Hospital such as exercise intolerance, nocturnal cough, loss of appetite, arrhythmia, early fatigue, shortness of breath, discomfort, cyanosis, abnormal lung sounds on lung auscultation-crackling, harharas, murmurs-, tachycardia, dyspnea, tachypnea. In the presence of clinical findings, lung radiogram, ECG (electrocardiogram) and ECO (echocardiography) were performed to diagnose dogs with suspected heart failure. This study; It was carried out with the approval of Kırıkkale University Animal Experiments Local Ethics Committee. (Date 29.09.2022, Number of Meetings: 2022/05, Decision No: 25). In clinically suspected dogs, first chest radiograph (ventrodorsal/ laterolateral) was taken for diagnosis. Afterwards, the patients were placed in the lateral position for ECO and ECG. Initially, bipolar electrodes were attached to the ECG electrodes, both forearms and left hind legs. Considering the anatomical position of the heart, one of the electrodes was placed on the right ventricle and the other two on the left ventricle on the fifth and sixth intercostal spaces. Standardization was printed by adjusting it to draw a curve of 10 mm height when 1 mV current was supplied to the device. ECG was

printed after the electrocardiography rate was set to 50 mm/s. Then, Mindray Vetus E7 color doppler ultrasound device and M-mode cursor were used. In order to obtain measurements and images, the hairs in the right and left parasternal (lower 1/3 of the right and left 3rd or 5th intercostal space) sections were shaved. The long axis line was checked from the right parasternal window (in the 3rd and 5th intercostal space for small breeds, and from the 5th and 6th intercostal space for large breed dogs). Images of the four chambers were taken and transversal imaging of the heart was performed to examine all structures from the apex to the base of the heart. The attachment sites of the left ventricle, chordae tendineae and papillary muscles were visualized. Eventually, the echo was completed by looking at the mitral and aortic valves. As a result of the examinations, in the patients; detected degenerative valve disease, mitral valve insufficiency, ACVIM class C heart failure, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), congestive heart failure (CHF). Dogs that were clinically suspected and diagnosed with heart failure as a result of echocardiography were included in the study group. Samples were made from these animals diagnosed with heart failure. At the same time, blood samples were taken from 10 healthy dogs (Control group) negative for HF and other diseases. Serum ADM levels were measured in blood samples taken from the diagnosed (n=20) and control group (n=10). In order to evaluate the adrenomedullin levels in animals in the study and control groups, 4 ml blood samples taken from Vena cephalica antebrachii were centrifuged for 10 minutes at 3500 rpm and serum samples were extracted into tubes with coagulation activating gel and enzyme-linked Immunosorbent Assay for Adrenomedullin (ADM), originating number CEA220Ca, was used to evaluate the adrenomedullin level in serum samples. The values obtained in the study were transferred to the computer environment and descriptive statistical information (mean, standard deviation, etc.) was obtained. The conformity of the measurement and scoring values to the normal distribution was examined graphically and with the Shapiro-Wilk test. Normal distribution was observed in all groups. Independent sample T test was used to evaluate the data in the study groups (Heart disease) (n=20) and the data in the Control group (Healthy) (n=10). As a result of the statistical tests,  $p < 0.05$  level difference was evaluated as significant.

## Results

Complete blood count, basic biochemical parameters and N-terminal pro-B type natriuretic peptide (NT-proBNP) neurohormone were measured in patients in the study group. Complete blood count results, (Leukocyte count (WBC), Erythrocyte count (RBC), Hematocrit count (HCT), Platelet count (PLT), Hemoglobin value (Hb), Mean Corpuscular Hemoglobin (MHC), Lymphocyte level (Lym), no significant increase or decrease in platelet width (PDW) values was encountered. Blood Urea Nitrogen (BUN-P), one of the biochemical parameters examined, was found to be high in 5 of 20 patients. No significant increases in creatinine (CRE-P III), gamma glutamyl transferase (GGT-P III) Alanine Aminotransferase (GPT/ALT-P), Total Bilirubin (T. BİL-P III) value was encountered. Total Protein (TP-P III) value was found to be high in 4 of 20 patients, Albumin (ALB-P) value was low in 3 of 20 patients and high in 2 patients, Alkaline phosphatase (ALP-P III) value was high in 4 patients, Total cholesterol concentration (TCHO-P III) value was measured high in 4 patients, c. NT-proBNP neurohormone measurement values were observed to be high in all but 3 patients and gave suspicious results.

**Table 1:** Serum biochemical analyse results

| Parameter          | Mean  | Min  | Max   |
|--------------------|-------|------|-------|
| BUN-P (mg/dl)      | 24.89 | 17   | 35.7  |
| TP-P III (g/dl)    | 6.89  | 5.1  | 11.2  |
| ALP-III (U/I)      | 77.61 | 30.8 | 236.8 |
| GGT-PIII (U/I)     | 9.88  | 3.8  | 14    |
| T.BİL-PIII (mg/dl) | 0.32  | 0.1  | 0.5   |
| ALB-P (g/dl)       | 5.49  | 2.5  | 24.8  |
| CRE-PIII (mg/dl)   | 1.27  | 0.48 | 2.32  |
| GPT/ALT-P (U/I)    | 46.7  | 16   | 78    |
| TCHO-PIII (U/I)    | 285.7 | 114  | 785   |

Ventrodorsal (V/D) and laterolateral (L/L) imaging of the thoracic region was performed with direct digital radiography in patients presenting with complaints of respiratory distress such as coughing, difficulty breathing, and shortness of breath. Radiographic results revealed an enlarged heart size, interstitial edema,

oval appearance at the anterior edge of the heart, increased contact of the heart with the sternum, and pleural effusion in the lung interstitium. As a result of ultrasonographic examinations, it was observed that the valves remained open in systole when viewed with 2D (2d).

Diastolic increase in left ventricular inside diameter and left atrial (LA) pressure was encountered. Intramural arterial narrowing and mitral leak were observed. When viewed in M-mode, left atrial dilatation and thickening of the mitral valve leaves were observed. Cardiac arrhythmia, continuous supraventricular extrasystoles, tachycardia, enlarged P wave, prolonged R wave were recorded in some patients on ECG. In this way, 10 patients were diagnosed with mitral valve insufficiency (50%) and 4 patients with right heart failure (20%). 5 patients had irregular mitral valve movements, LA dilatation, decreased atrial ejection fraction (EF), progressive atrial fibrillation (AF) and left ventricular dilatation. AF enlarged, in addition to these symptoms, an increase in left ventricular (LV) diameter was observed in 2 (10%) patients. In the ECG, rare ventricular arrhythmias, cardiac arrhythmias, atrial fibrillation, irregular R wave, abnormal QRS complex and prolonged QRS complex were noted in 3 patients (15%). QRS complex was recorded as small in 2 (10%) patients.

## Discussion

Adrenomedulline (ADM) is a compound originally isolated from extracts of human pheochromocytoma tissue, has been identified as a neurohumoral factor with potent vasodilatory activity. (Kanno, et., 2012). Kanno et al. (2012) reported that ADM, a peptide determined to have vasodilator and natriuretic effects, plays a role in the regulation of the cardiovascular system. Although systolic heart failure cases in dogs are encountered quite frequently when considering the population, the definitive diagnosis is quite difficult. However, there are no studies designed to evaluate the characteristics and outcomes of the disease from diagnosis to treatment. In this case, veterinarians have difficulties in the diagnosis and treatment of the disease as a result of the lack of significant studies on systolic heart failure. (Schober et. al., 2022). Cases of heart failure observed in older dogs are quite common in clinics. As a clinical sign in all heart failure, it is seen together with the symptoms seen in left and right ventricular heart failures. Clinical findings of heart failure; While cough and tachypnea are symptoms we encounter in left heart failure, they are the first symptoms that occur in exercise intolerance.

rance failure (Aytekin, 2009). The most common manifestation of right heart failure (R-CHF) in dogs is ascites; Other sites of fluid accumulation (which may occur together) are pleural effusion, small-volume pericardial effusion (without tamponade), and rarely peripheral edema (DeFrancesco ve Ward, 2021). Onmaz et al. (2011) in their research, . They stated that they encountered clinically dyspnea, cough, fainting, inability to tolerate exercise, abdominal tension and partial anorexia, and moderate lethargy with weight loss. In this research, the control group was formed with animals brought to their clinics for control, sterilization and vaccination. In the blood count, laboratory results and physical examinations, all vital signs were observed to comply with the healthy animal profile. Clinical signs detected in dogs with heart failure (n=20) without gender and breed predisposition included in the study group; fatigue, cough, shortness of breath, difficulty sleeping, weight loss, fainting, respiratory distress, cyanosis in the mucous membranes were detected. Although these clinical signs vary in each animal, they were found to be consistent with the studies of Aytekin (2009), Onmaz et al. (2011) and DeFrancesco and Ward (2021). Ok et al. (2010) found atrial fibrillation in one dog and ventricular tachycardia in two dogs in their study on three dogs with congestive heart failure. On radiographs, they noted an overgrowth of the heart (approximately eight ribs wide), and on ultrasonographic examination they detected enlargement of the ventricles and fluid in the heart sac and abdominal cavity. Brownlie and Cob (1999), in their 10-year study, found that eighteen dogs with CHF had atrial fibrillation (AF) at their first visit. Other ECG abnormalities detected were ventricular and supraventricular premature contractions, first and second degree atrioventricular block, P mitral and left anterior fascicles, and right bundle branch blocks. In this research, from dogs with heart failure (n=20); Ventricular tachycardia was detected in 4 patients (20%), atrial fibrillation was detected in 5 patients (20%). Right bundle branch blocks were recorded in 3 patients (15%). In the radiographic examination, an enlarged heart size was observed in 12 patients (60%). In the ultrasonographic examination, left ventricular dilatation was noted in 5 (20%) patients. These findings are similar to the findings observed in the studies of Brownlie and Cob (1999) and Ok et al. (2010). In dogs with dilated cardiomyopathy, systolic and

diastolic inner diameters of the left atrium or ventricle, M-mode echocardiographic abnormalities have been reported due to the distance between the mitral valve E point and the interventricular septum and, if the right ventricle is affected, the increase in the right ventricular inner diameter together with the decrease in ventricular contraction force (hypokinesia). ECG results of dogs with significant heart failure due to DCM mostly show left ventricular enlargement, high amplitude R wave, enlarged QRS complex, and enlarged P wave. (Onmaz, et al., 2011). In our research, 5 patients had irregular mitral valve movements, dilatation of the LA, decreased EF, progressive atrial fibrillation (AF) and left ventricular dilatation. In addition to these symptoms, an increase in LV diameter was observed in 2 patients (10%). Rarely, ventricular arrhythmias, cardiac arrhythmias, atrial fibrillation, irregular R wave, abnormal QRS complex and prolonged QRS complex were noted in 3 patients (15%) on ECG. QRS complex was recorded as small in 2 patients (10%). Gönül et al. (2017) looked at the results of hematological and biochemical analysis in their study on 10 cardiac dyspnea, 10 respiratory dyspnea and 10 healthy dogs. The average results of the analyzes performed in dogs with cardiac dyspnea and respiratory dyspnea, respectively; Urea (mg/dL): 29.75 - 24.25, Creatinine (mg/dL): 0.82 - 0.85, ALT (IU/L): 40.25 - 44, ALP (IU/L): 58.75 - 64.25, - GGT (IU/L) ): 4.75 - 4.25, Cholesterol (mg/dl): 209.75 - 237.50 recorded and stated that they did not see a statistically significant difference between the groups in terms of the results of this analysis. In our results, hematological and biochemical analyzes were examined in 20 dogs with heart failure. In the biochemical analyzes examined; BUN-P (mg/dL) mean 24.885 (min. 17 - max. 35.7) TP-P III (g/dL) mean 6.886 (min. 5.1 - max. 11.2), ALP-III (IU/L) mean 77.605 (min. 30.8 - max. 236.8), GGT-P III (IU/L) mean 9.88 (min. 3.8 - max. 14), T. BİL III (mg/dL) average 0.32 (min. 0.1 - max. 0.5), ALB-P (g/dL) average 5.485 (min. 2.5 - max. 24.8), CRE-P III (mg / dL) average 1.27 (min. 0.48 - max. 2.32), GPT/ALT-P (IU/L) average 46.7 (min. 16 - max. 78), TCHO-P III average Recorded as 285.7 (min. 114 - max. 785). There was no significant change in the hematological analysis results of healthy dogs in the control group (n=10). These results support the work of Gönül et al. Gönül et al. (2017) reported that measuring NT-proBNP can distinguish clinical symptoms such as dyspnea, coughing, pulmonary edema from non-cardiac respiratory stress from cardiac origin, determining the severity of heart disease, estimating the onset of congestive heart failure, dilated cardiomyopathy and myxomatous stated that it can be helpful and useful

in providing predictive information in mitral valve diseases and in making treatment decisions. Raffan et al. (2009) reported that N-terminal pro-B-type natriuretic peptide (NT-proBNP) is used as a prognostic indicator in human medicine and to monitor the progression of the disease to distinguish between cardiac and non-cardiac causes of dyspnea, and studies have also shown that NT-proBNP has recently been observed to increase in dogs with heart disease. In this research, NT-proBNP values were examined in dogs with CHF. The mean NT-proBNP (pmol/L) values were calculated as 1105,565 (min. 382.7 - max. 1919.1) and it was observed that the NT-proBNP value increased compared to healthy dogs. On the other hand, NT-proBNP value was not found to be at risk or abnormal values in the control group, and these findings were found to be similar to the findings of Raffan et al.'s (2009) study. Jougasaki et al. (2001) stated that ADM and ADM receptor system increased in the rat ventricle during postinfarction heart failure. Etöz and Büyükcöskün (2005) observed that the plasma concentration of ADM increases in various cardiovascular diseases. Likewise, Voors et al. (2019) reported that the amount of ADM is strongly elevated in patients with acute heart failure. In this research, it was observed that adrenomedullin (ADM) levels measured in blood serum increased in dogs diagnosed with heart failure, in line with the above-mentioned studies. Kanno et al. (2012) found insufficient data on circulating ADM concentrations in animals with mitral regurgitation. Likewise, high plasma concentrations of ADM have been reported in people with congestive heart failure (CHF). They found few reports of ADM concentrations in canine heart failure samples. Circulating ADM concentrations in dogs with CHF are still not determined and it is unknown whether ADM has the potential to be a useful biomarker in the diagnosis of dogs with CHF. In this research, it was determined that ADM level was high in 10 patients (50%) with mitral valve regurgitation, which supports the literature. Jougasaki et al. (2001) reported that experimental CHF induced by pacing showed activation of circulating and ventricular ADM in an animal model by radioimmunoassay, immunohistochemistry, in situ hybridization and Northern blot analysis in their study on 17 male dogs with heart failure. Immunohistochemistry and in situ hybridization noted increased ADM expression in ventricular myocytes associated with increases in ventricular ADM concentrations determined by radioimmunoassay. In our research, ADM levels were examined in 20 dogs

with congestive heart failure (CHF) and an increase in ADM concentrations in the blood was detected. Haehling et al. (2010) stated in their study that the prognostic value of NT-proBNP is lower in people with heart failure compared to MR-proADM. They stated that NT-proBNP value was not a supportive prognostic factor compared to MR-proADM as the basic model. In this research, the NT-proBNP values of the patients in the study group were evaluated. It was observed that NT-proBNP value increased in proportion to the increased ADM value in dogs with heart failure. Hinson et al. (2000) argued that it is not yet known whether systemic increases in adrenomedullin reflect an overflow from local production and sites of action, or whether increased plasma adrenomedullin under certain conditions has a hormonal function that causes an overall decrease in vascular resistance and a decrease in blood pressure. Haehling et al. (2010), They argued that MR-proADM is an indicator of mortality in patients with CHF, regardless of established markers such as low left ventricular ejection fraction (LVEF), New York heart society (NYHA) class, creatinine, age, or NT-proBNP. Hinson et al. (2000), They argued that elevated adrenomedullin is a consequence rather than a cause of pathology, and it is unclear whether systemic increases in adrenomedullin reflect an overflow from local production and sites of action, or whether increased plasma adrenomedullin under certain conditions has a hormonal function that causes a decrease in overall vascular resistance and blood pressure. Voors et al. (2019), suggested that a new immunoassay has been developed that specifically measures biologically active ADM (bio-ADM) and that this immunoassay could become a biomarker for ADM, tissue occlusion. The most promising clinical application of ADM is the evaluation of proadrenomedullin at the plasma level as a biomarker in cardiovascular diseases. Proadrenomedullin level is a good predictor for prognosis and survival in patients with coronary artery disease or heart failure (Beetowski and Jamroz, 2004). İcen et al. (2009), They mentioned that there is no blood test to make a definitive diagnosis of heart failure, but that hormones and enzymes in the circulation, called biomarkers, can be a biomarker that can be used in the diagnosis of heart failure. They also stated that there are two important test types that show myocardial cell degeneration in the pathophysiology of heart patients, and they are neurohormonal or inflammatory markers.

## Conclusion

This research, supports that ADM, which is also defined as a neurohumoral factor with strong vasodilator activity, has a potential role in neurohumoral activation in the progression of CHF. As a result, it is obvious that there are still difficulties in the diagnosis and follow-up of heart failure observed in dogs in the veterinary field. Although this study suggests that serum ADM measurements may shed light on the diagnosis of heart failure in dogs and the pathogenesis of the disease, it was concluded that further scientific studies are needed in the future.

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